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Application of computational toxicological approaches in human health risk assessment. I. A tiered surrogate approach

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abstract

Hazard identification and dose–response assessment for chemicals of concern found in various environmental media are typically based on epidemiological and/or animal toxicity data. However, human health risk assessments are often requested for many compounds found at contaminated sites throughout the US that have limited or no available toxicity information from either humans or animals. To address this issue, recent efforts have focused on expanding the use of structure–activity relationships (SAR) approaches to identify appropriate surrogates and/or predict toxicological phenotype(s) and associated adverse effect levels. A tiered surrogate approach (i.e., decision tree) based on three main types of surrogates (structural, metabolic, and toxicity-like) has been developed. To select the final surrogate chemical and its surrogate toxicity value(s), a weight-of-evidence approach based on the proposed decision tree is applied. In addition, a case study with actual toxicity data serves as the evaluation to support our tiered surrogate approach. Future work will include case studies demonstrating the utility of the surrogate approach under different scenarios for data-poor chemicals. In conclusion, our surrogate approach provides a reasonable starting point for identifying potential toxic effects, target organs, and/or modes-of-action, and for selecting surrogate chemicals from which to derive either reference or risk values.

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1. Introduction

Although computational toxicological or in silico approaches have been used commonly in ecological risk assessment or pharmaceutical drug safety assessment or screening, they are rarely used for characterizing dose–response relationships in human health risk assessment. Since the release of the National Academy of Sciences (NAS)' Toxicity in the 21st Century: A Vision and a Strategy (2007) and Science and Decision: Advancing Risk Assessment (2008), there has been a growing interest in a paradigm shift to rely more on the recent advances in technologies and tools to predict and assess toxicity for environmental chemicals. One of the technologies identified in Chapter 4 of Toxicity in the 21st Century (NAS, 2007) is structure–activity relationship (SAR). SAR is a means by which the effect of a toxic chemical on an animal, a human, or the environment can be related to its molecular structure. Traditionally, this type of relationship may be assessed by considering a series of chemicals, making gradual changes to their structures (e.g., functional groups), and noting the effect of each change on their biological activity (Tong et al., 2003). Alternatively, it may be possible to assess similarity by using software programs or models to establish a structure–activity relationship among the chemicals. Many publicly accessible programs can provide quantitative assessment (similarity scores) for identifying and ranking potential structural analogs.

Because animal testing is often expensive and time-consuming, alternative methods relating chemical structure to biological activity or toxicity have become increasingly valued in many regulatory settings (e.g., Registration, Evaluation, Authorisation and Restriction of Chemical substances [REACH], http://ec.europa.eu/environment/chemicals/reach/reach_intro.htm). By improving the current methods in predicting toxicological endpoints (or target organs) and chemical mechanisms of toxicological endpoints, and by compiling toxicological data for user-friendly data-mining (e.g., digitize legacy documents), risk assessors can be better equipped to address potential human health adverse effects, and to provide probabilistic (surrogate) risk values for data-poor chemicals. For highly regulated substances (e.g., drugs, pesticides, etc.), SAR is rarely a stand-alone approach for the purpose of hazard identification and dose–response assessment, and experimental validations often follow. However, for environmental chemicals with regulatory implications, the SAR surrogate approach has greater responsibility, because further animal testing may not be available for validating the SAR-based toxicity prediction. Often, the SAR surrogate approach alone provides a "screening level" but scientifically justifiable risk value with policy

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implications. Therefore, the strength of the SAR surrogate approach often depends on the available toxicity data, with heavier emphases on commonality of target organ, toxic effect, and/or mode-of-action (MOA) among the potential surrogates to provide a strong support in addition to the chemical structure similarity.

The existing guideline on application of SAR to risk assessment of environmental chemicals is described in US EPA's Guidelines for Carcinogen Risk Assessment (2005), but it is mostly limited to qualitative assessment and cross-chemical class comparison of carcinogenic potential. However, SAR in general has been proven to be a useful tool for pharmaceutical risk assessment and rational drug design to derive a probabilistic toxicity value for unknown chemicals or candidate drugs (Bodor, 1999; van den Broek et al., 1989; Congiu et al., 2004). Furthermore, in a recent article by Wu et al. (2010), the authors provide a comprehensive framework for SAR-based toxicological assessments for both pharmaceutical and industrial chemicals. Wu et al. (2010) demonstrate a systematic approach for analyzing and evaluating suitability of analogs for consideration in SAR-based toxicological assessments, which plays an important role in the hazard identification step of risk assessment. To take advantage of the knowledge learned and tools described in previous examples, a tiered approach based on SAR and read-across (collectively referred to as surrogate approach), which can include chemical structure, physicochemical properties, MOA, pharmacokinetics, (bio)degradation, environmental fate and transport, target organ, and many other experimental and theoretical parameters, is proposed herein for assessing environmental chemicals quantitatively, extending beyond hazard identification to dose–response assessment. We believe that by taking into considerations of chemical (structure) and biological (metabolism and activity), a surrogate approach using the available data and sound rationales can be applied to provide a reasonable prediction.

Our tiered surrogate approach specifically focuses on three types of surrogate chemicals (hereon referred to as surrogates): (1) structural, (2) metabolic, and (3) toxicity-like. This approach is similar to "suitable analogs" as defined in OECD Series on Testing and Assessment No. 80 Guidance on Grouping of Chemicals (OECD, 2007), previously proposed for assessing ecotoxicity. "Suitable analogs" can be based on either chemically (e.g., chemical structure) or biologically (e.g., metabolic breakdown) similar chemicals. Given the fact that "suitable analogs" were mostly used in the context of ecological risk assessment previously, we coined "surrogate" in the context of human health risk assessment and further expanded to the three types of analogs (surrogates) with broader definitions (see below).

The structural surrogates may include similarities in structure and reactivity, defined by structural alerts, key functional groups, etc. (Ashby, 1985; Sanderson and Earnshaw, 1991; Wu et al., 2010), or by predefined chemical grouping or categories (Cronin et al., 2011). Different databases and similarity algorithms may be applied (e.g., ChemIDplus available from http://chem.sis.nlm.nih.gov/chemidplus/, DSSTox available from http://www.epa.gov/ncct/dsstox/index.html, OECD QSAR Toolbox available from http://www.oecd.org/document/23/0,3746,en_2649_34379_33957015_1_1_1_1,00.html, AMBIT available from http://ambit.sourceforge.net/, and ToxMatch available from http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxmatch) to identify structural surrogates that may have repeated dose toxicity values (either manually or via a predefined toxicity dataset/database). However, due to lack of a centralized federal risk assessment data repository for regulatory considerations (i.e., peer-reviewed and vetted human health toxicity values) and searchable functionality (i.e., not structure-searchable), the searching software programs of structural surrogates were limited to ChemIDplus and DSSTox. Without the structure data files and toxicity values to be inputted into other software programs, similar chemicals with the available vetted toxicity values have to be manually searched or verified.

The second type of surrogates may include metabolic precursors, metabolites, and (bio)degradation products/precursors. The target organ(s)/tissue(s) should be noted for the potential surrogates, especially if the target organ for the chemical of concern is known (or predicted by validated classification models). Environmental fate and transport for exposure assessment may also be considered if the chemical of concern could be subjected to significant weathering and (bio)degradation (e.g., breakdown or by-products of a parent compound may be considered as potential surrogates). Such information may be obtained through a literature search or toxicokinetic testing. Potential metabolic surrogates and the chemical of concern are expected to have similar toxicological profile or mode(s) action that may result in ultimate toxicity at the same target organ(s) or tissue(s).

The third type of surrogate, toxicity-like, is commonly found in well-defined chemical mixtures where either a toxicity equivalent factor (TEF) or relative potency factor (RPF) has been established for an index chemical and the rest of a mixture (e.g., dioxins, polycyclic aromatic hydrocarbons, etc.; USEPA, 1993, 2000, 2010). These chemicals can be structurally similar and are often found to have similar dose–response curves for either one or all endpoints (RPF or TEF, respectively). Other well-validated in vitro dose–response data can be considered as well for establishing relative potency ranking.

It is important to note that all potential surrogates from any of the three types need to have toxicity information from repeated dose exposure for further consideration (i.e., long term dose–response relationship). Finally, a weight-of-evidence approach is applied to the pooling of these three types of potential surrogates, the ultimate goal being selection of a surrogate that is likely to be the most toxicologically relevant to the chemical of concern. Overall, the main concept for conducting the tiered surrogate approach assessment proposed herein is to find all types of potential surrogates that may result in similar toxicological profiles. Then, with sufficient scientific justification, the toxicity values of the most appropriate surrogate can be suggested as the surrogate toxicity values for the chemical of concern.

2. Materials and methods

2.1. Tiered surrogate approach

The tired surrogate approach can be illustrated using a decision tree; it is presented in Fig. 1. A general protocol for conducting a tiered surrogate approach for a hypothetical chemical is presented as follows:

Starting with a chemical of concern (top of Fig. 1), the initial step is to identify any appropriate in vivo experimental or epidemiological data (repeated dose toxicity information) in literature for that chemical. If such data exist, follow the established risk assessment paradigm for dose–response using a no-observed-adverse-effect level/lowest-observed-adverse-effect level (NOAEL/LOAEL) or lower confidence limit on the benchmark dose (BMD/BMDL) approach to define the point of departure (POD) for deriving a reference value. If no such data exist, prior to starting with Step 1 of the surrogate approach, conduct a literature search to determine if the parent compound could be transformed (either biologically or chemically) to either a breakdown product(s) or major metabolite(s) that is ultimately responsible for the manifested toxicity. Such examples can be hydrolysis products or enzyme-mediated biotransformed products. If a major toxic moiety such as a major metabolite with a delineated metabolic pathway is known to cause the adverse effect and toxicity observed at the organism level, use the direct toxicity information (repeated dose study) of the major metabolite in the traditional risk assessment approach. Similarly, if the major toxic moiety is a hydrolysis product, then use the

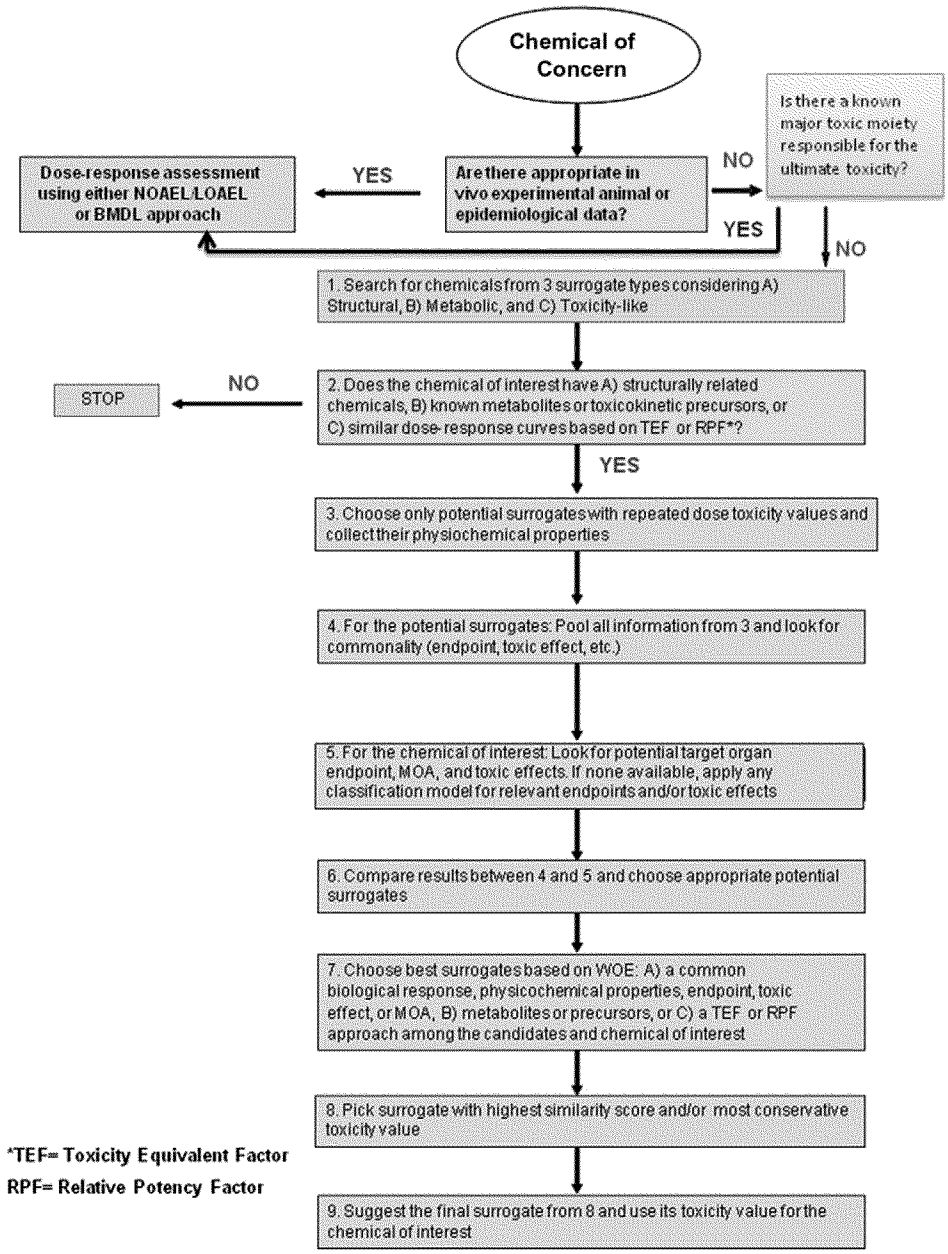


Fig. 1. Tiered surrogate approach.

toxicity information of the hydrolysis product instead. If this is not the case, start with Step 1 of the surrogate approach.

In Step 1 of our tiered strategy, identify any candidates (potential surrogates) from the three surrogate types and select them based on available repeated dose toxicity values. In Step 2, determine whether the chemical of concern has (A) structurally related chemicals, (B) known metabolites (intermediate or final) or toxicokinetic/degradation precursors, or (C) similar dose–response curves based on a TEF or RPF[1]. If no candidates could be identified, then the tiered surrogate approach cannot be applied. If candidates could be identified, continue on to Step 3. In Step 3, choose only candidates in any of the three types that have repeated dose toxicity values and collect all relevant information such as physiochemical properties and any in vitro and toxicokinetic data. In Step 4, combine all candidates into a potential surrogate list containing all the relevant information. From this list of potential surrogates, look for commonality such as similar endpoint(s), toxic effect(s), or if possible, common (bio)degradation pathway(s), environmental fate and transport. At this step, if only the structural surrogates based on structural similarly alone (e.g., no chemical-class specific information, etc.) are available and without any biological/toxicological understanding that can be inferred about their potential activity or toxicity, we strongly advise that no further steps be taken. In other words, the surrogate approach may not be suitable for the chemical of concern due to insufficient information.

In Step 5, look for a potential target organ, endpoint, MOA, or toxic effect on the chemical of concern itself (as similarly done

[1] TEF and RPF are defined in US EPA 2000 Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures (EPA/630/R-00/002 – August 2000): "The RPF method uses empirically derived scaling factors that are based on toxicity studies of the effect and exposure conditions of concern in the assessment. When extensive mechanistic information shows that all the toxic effects of concern share a common mode of action, then one scaling factor is derived for each chemical that represents all toxic effects and all exposure conditions. This special case is the TEF method, where actual toxicologic equivalence between the component chemicals is assumed once the scaling factor is applied."

for the potential surrogates in the previous step). If none of the aforementioned information can be located, then apply any commercial (e.g., DEREK, Lhasa Limited) or open-source classification or prediction models to estimate relevant endpoints (e.g., liver toxicant) and/or toxic effects (e.g., necrosis) for the chemical of concern.

In Step 6, compare the results between Steps 4 and 5 and choose appropriate potential surrogates based on an overlap of toxicity information among the potential surrogates and chemical of concern (e.g., is there a chemical class-specific effect?). Finally, pool information from Steps 4–6 and add them to the existing information from Step 4 for both the chemical of concern and selected potential surrogates. In Step 7, use a weight-of-evidence approach to further filter the chosen potential surrogates based on the following rationales: (A) is there a common or similar biological response, toxic effect, endpoint, target organ, or MOA among the chemical of concern and potential surrogates? (B) Is there any metabolite(s) or precursor(s) (including [bio]degradation products) that may lead to the observed toxicity? (C) Is there a TEF or RPF approach among the potential surrogates and chemical of concern? During this step, more emphasis will be given to toxicological similarity over structural similarity. At this step, there should be a short list of potential surrogates with commonality based on the weight of evidence (WOE) (from the rationales A, B, or C). In this step, some potential surrogates should be excluded if they do not have any WOE-defined commonality or have significantly different physicochemical properties (e.g., solubility, pK_a) and toxicokinetic profiles that set them apart from the rest of potential surrogates and chemical of concern.

Next, in Step 8, select the best surrogate with highest similarity score (structural similarity) and/or most conservative toxicity value (e.g., lowest for non-cancer effects or highest for cancer potency) from the remaining potential surrogates. If there are multiple common endpoints, use the most sensitive endpoint. Finally, in Step 9, suggest the surrogate selected from Step 8 and use its toxicity value for the chemical of concern and clearly document any known uncertainty and limitations of the surrogate toxicity value.

3. Results

3.1. Scenarios that do not require the tiered surrogate approach—direct surrogates

Prior to conducting a comprehensive tiered surrogate approach, one should look at the available literature studies and determine if there are straightforward substitutes or direct surrogates that could ultimately be responsible for the observed toxicity at organism level via either chemical (e.g., hydrolysis)- or bio-transformation (e.g., enzyme-mediated). There are two cases that fall into this category: methyl acetate (see below) and allyl alcohol (see Supplemental Data).

Methyl acetate (CASRN: 79-20-9) is hydrolyzed to methanol, as was shown for glycol ether acetates that are hydrolyzed to their parent alcohols in aqueous solution (Miller et al., 1984, 1983; Nagano et al., 1979). In vitro hydrolysis of methyl acetate is a reversible reaction; two products of this reaction are methanol and acetic acid as follows (Mizunuma et al., 1992):

$$MeAc + H_2O \rightleftharpoons HAc + MeOH \quad (1)$$

Hendersen and Haggard (1943) suggested that the methanol formed by hydrolysis of methyl acetate in the human body might be responsible for its toxicity. Two studies in humans and rabbits, respectively, indicate that methanol is produced in both species respectively following exposure to methyl acetate (Tada et al., 1974; Tambo, 1973). Therefore, it is reasonable to use the POD (no-observed-effect level [NOEL] of 500 mg/kg-day; http://www.epa.gov/ncea/iris/subst/0305.htm) of methanol as the POD for methyl acetate after molecular weight (MW) adjustment (74.08 / 32.04 g/mol = 2.312), which is approximately 1160 mg/kg-day. One can then apply a composite uncertainty factor (UF_C) for the methanol assessment to the adjusted POD (based on the surrogate, methanol) to derive a reference dose (RfD) for methyl acetate. It should be noted that such a surrogate value has higher uncertainty, because potential toxicity due to acetic acid (another hydrolysis product), especially at high exposure, is not directly accounted for (see full analysis in http://hhpprtv.ornl.gov/issue_papers/MethylAcetate.pdf).

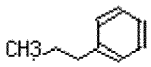
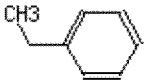
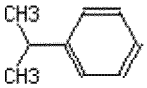
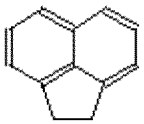
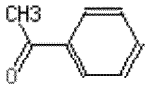
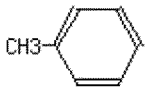
Similar to the example of methyl acetate above, the toxic effects of allyl alcohol exposure can be attributed to acrolein (NTP, 2006), and the POD (a NOAEL of 0.05 mg/kg-day) for acrolein can be used as the surrogate POD for allyl alcohol assessment (See Supplemental Data). In summary, methyl acetate and allyl alcohol do not need to go through the tiered surrogate analysis (decision tree in Fig. 1). However, such quantitative information on the parent and major metabolites and their ultimate toxicity is often not known, which makes the direct surrogate approach impossible. Nevertheless, their trend and profiles can be compared, which is the basis for the metabolic surrogate in the Section 3.2.

3.2. Chemical risk assessment of n-butylbenzene—a test case study of surrogate approach

n-Butylbenzene (CASRN 104-51-8) is a high production volume chemical which is a component of linear and branched butylbenzene mixtures (CASRN 68411-44-9) for which there is a need to develop a risk value for regulatory purposes (http://www.epa.gov/HPV/pubs/update/hpv_1990.htm). At the time of a previous assessment, there were no subchronic or chronic toxicity studies on n-butylbenzene. Due to the lack of toxicity information to derive a risk value for human health risk assessment, a structure activity relationship (SAR)-based surrogate approach was applied to derive a non-cancer risk value for n-butylbenzene. During reassessment, a two-generation reproductive toxicity study of n-butylbenzene by Izumi et al. (2005) was used to evaluate our previously estimated non-cancer risk value based on the surrogate approach. In this test case study, both surrogate and traditional approaches are presented and compared. This is the first demonstration study that the tiered surrogate approach has proven to be reasonably predictive.

Prior to initializing the surrogate approach, an attempt was made to search for a major toxic moiety that results in the final observed toxicity (e.g., a natural breakdown product or major metabolite). This step is important because a parent compound could be biotransformed or modified to a major metabolite(s) or breakdown product(s) that is ultimately responsible for the manifested toxicity. There is no such toxic moiety found for n-butylbenzene, therefore the surrogate approach was applied. There are three types of potential surrogates that may be suitable for the SAR-based dose–response assessment as part of human health risk assessment. First, starting with the structural surrogates, a similarity search using ChemIDplus (a 2D + 3D similarity search) was performed using n-butylbenzene as the query chemical. However, none of the hits had repeated dose information. Subsequently, DSSTox (a 2D similarity search), another open-source similarity program, was used to broaden the search for locating other suitable structural surrogates. Only those hits with repeated doses are considered as potential structural surrogates. As a result, only 5 hits (their similarity score expressed in percentage) were found on the IRISTR_v1b database within DSSTox: ethylbenzene (70%), isopropylbenzene (or cumene; 67.7%), acenaphthene (57.7%),

Table 1
Results of DSSTox potential structural surrogates.

	n-Butylbenzene	Ethylbenzene	Cumene	Acenaphthene	Acetophenone	Toluene
Structure						
Similarity	100 (query)	70	67.7	57.7	53.8	50

acetophenone (53.8%), and toluene (50%). Table 1 provides the structural information on these chemicals (see Supplemental Data for full analysis).

Although other similarity algorithms or SAR-based software programs can be used, we only focused on ChemIDplus and DSSTox, because these two publically accessible programs provide numerical (quantitative) similarity ranking that is transparent and reproducible and can be used in the later step for WOE consideration. Notably, they also do not require a predefined toxicity dataset (e.g., long term mammalian toxicity) to compare chemicals structurally and then check for their availability of repeated dose information (it is currently done by cross-checking US EPA's IRIS [http://www.epa.gov/iris], PPRTV [http://hhpprtv.ornl.gov], and other federal toxicity databases).

Three out of five of the hits are short-chained alkylbenzenes (i.e., toluene, ethylbenzene, and cumene), and this chemical class has comparable physicochemical properties (e.g., boiling point, density, etc.). The solubility in water normally decreases with the number and length of the side-chain group substituted on the benzene ring. Decrease in volatility also correlates with increasing MW because of the lengthening of the alkyl chain (Gerarde, 1959; Table 2). Among these five candidate chemicals, acenaphthene and acetophenone were excluded for further consideration because of their unique structural properties that differentiate them from the general alkylbenzenes (see Table 1). For example, acenaphthene has three fused rings that give the chemical a completely different resonance and electrophilicity profile from the alkylbenzene class. Acetophenone has a differing acetyl functional group which would change the toxicity profile of a chemical due to changes in toxicokinetics. This is consistent with the effects of additional functional groups that may change reactivity or toxicity as mentioned by Wu et al. (2010). Finally, only 3 hits (ethylbenzene, cumene, and toluene) were retained and considered as the structural surrogates.

Next, in order to look for potential metabolic surrogates for n-butylbenzene, a literature search on any metabolic or toxicokinetic aspects for alkylbenzenes was conducted. Because alkylbenzenes in general have chemical class specific information (e.g., similar metabolic profile information; see details below), the search was limited to the low carbon-number alkylbenzene class only (e.g., n-butylbenzene is a C10 alkylbenzene). In other words, we attempted to verify the suitability of these 3 structural surrogates as potential metabolic surrogates without further consideration of other potentials outside of the alkylbenzene class (note: this may not be suitable for other chemicals of concern that do not have this kind of class specific information).

The available information on the absorption and elimination of some alkylbenzenes suggest that alkylbenzenes are in general readily absorbed and excreted, primarily in the urine (El Masry et al., 1956; Sećzuk and Litewka, 1976; Research Triangle Institute, 1989). Table 3 presents a comparison of the available oral absorption data and elimination profiles.

Gerarde (1959) stated that there is a common metabolic pathway for the alkylbenzenes (including ethylbenzene, cumene, n-butylbenzene, etc.) in rats. Gerarde and Ahlstrom (1966) stated that there could be a dual metabolic pathway of side-chain oxidation and ring hydroxylation, with the former preferred in rats. These three alkylbenzenes (i.e., toluene, ethylbenzene, and cumene) showed a similar toxicokinetic profile in comparison to n-butylbenzene, indicating the suitability of being the metabolic surrogates.

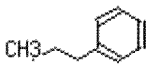
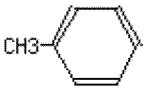
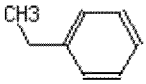
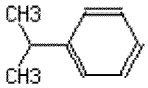
Cytochrome P450 (CYP) enzymes in general are responsible for the metabolism of the alkylbenzenes, another way to evaluate metabolic consequences or outcomes after longer exposure to alkylbenzenes is to examine the CYP enzyme inducibility and their potential effects on metabolite formation (i.e., secondary pathway via aromatic hydroxylation). Imaoka and Funae (1991) demonstrated that various n-alkylbenzenes (i.e., n-butylbenzene, ethylbenzene, and toluene [cumene was not examined]) induce hepatic enzymes (Table 4). There was a strong trend that the CYP inducibility is dependent on side-chain length and hydrophobicity. However, there is no pharmacokinetic data to compare n-butylbenzene with cumene. Given the available CYP induction profiles, ethylbenzene has the closest CYP inducibility when compared to n-butylbenzene. Based on the available toxicokinetic information and inducibility of CYP isoenzymes presented in Tables 3 and 4 and above, these three structural surrogates (i.e., toluene, ethylbenzene, and cumene) can be considered as metabolic surrogates as well, with ethylbenzene being the most suitable (i.e., having the most similar toxicokinetic profile to n-butylbenzene).

Because there is no existing or established TEF or RPF for alkylbenzenes, no attempt was made to search for toxicity-like surrogates (the third type of surrogate in the decision tree). Following the proposed surrogate decision tree considering the three types of surrogates (structural, metabolic, and toxicity-like), three short-chain alkylbenzenes fulfilled the criteria and were selected as the final potential surrogates: toluene, ethylbenzene, and cumene. All three potential surrogates have acute toxicity values and chronic PODs on the IRIS database, and all relevant toxicity information is summarized in Table 5.

In summary, based on structural similarities and available PODs on the IRIS database, toluene, ethylbenzene, and cumene were identified as structural surrogates. The physical/pharmacokinetic/toxicokinetic information among several alkylbenzenes and their capabilities in induction of CYP enzymes in rat liver (see Tables 1–4) indicates that toluene, ethylbenzene, and cumene were suitable metabolic surrogates for n-butylbenzene. There are no existing or established TEF or RPF values for alkylbenzenes. No toxicity-like surrogates were found. Thus, toluene, ethylbenzene, and cumene were the final potential surrogates.

Data on the toxicities of n-butylbenzene for comparison with toluene, ethylbenzene, and cumene were limited to acute lethality studies (prior to publication of a two-generation reproduction toxicity study by Izumi et al., 2005). These acute toxicity data may not be used to assess long term toxicity values but may infer ranking in toxicity similarity and may suggest common target organs(s) and/or effects. Table 5 contains acute oral toxicity values (LD_{50}s) and mortality data for n-butylbenzene, toluene, ethylbenzene, and

Table 2
Physical properties of n-butylbenzene and potential sturctural surrogates.[a]

Chemical	n-Butylbenzene	Toluene	Ethylbenzene	Cumene
Structure				
CASRN	104-51-8	108-88-3	100-41-4	98-82-8
DSSTox similarity	100%	50%	70%	67.70%
Molecular formula	$C_{10}H_{14}$	C_7H_8	C_8H_{10}	C_9H_{12}
Molecular weight	134.22	92.14	106.16	120.19
Melting point (ffC)	└ 87.9	└ 94.9	└ 94.9	└ 96.03
Boiling point (ffC)	183.3	110.6	136.1	152.39
Vapor pressure (mmHg) at 25 ffC	1.06	28.4	9.6	4.5
Henry's Law Constant (atm-m^3/mole) at 25 ffC	0.016	0.0066	0.0079	0.0115
Water solubility (mg/L) at 25 ffC	11.8	526	169	61.3
LogK_{ow}	4.38	2.73	3.15	3.66

[a] ChemIDPlus (2010).

Table 3
Comparative absorption data for n-butylbenzene and potential surrogates.

Chemical	Route	Species	Absorption	Basis	Reference
Toluene	Oral	Rabbit	74%	Elimination of metabolites in urine (hippuric acid)	El Masry et al. (1956)
Ethylbenzene	Oral	Rabbit	73–83%	Elimination of metabolites in urine (hippuric acid, methylphenylcarbinyl glucosiduronic acid, and phenaceturic acid)	El Masry et al. (1956)
n-Butylbenzene	Oral	Rabbit	68–78%	Elimination of metabolites in urine (hippuric acid, phenylpropyl- and methylphenethyl-carbinylglucuronides and phenaceturic acid)	El Masry et al. (1956)
Cumene (isopropylbenzene)	Oral	Rat	P 70%	Elimination of metabolites in urine (2-phenyl-2-propanol and its glucuronide or sulfate conjugates, and conjugates of 2-phenyl-1,2-propanediol)	Research Triangle Institute (1989)

Table 4
Induction of cytochrome P450 isoenzymes in rat liver by n-alkylbenzenes.[a]

	Changes in the levels of cytochrome P450[b]			
P450 isoenzyme	Control	Toluene	Ethylbenzene	n-Butylbenzene
IIB1	<0.5	10.4 ± 2.3[c]	30.9 ± 0.4[c]	52.8 ± 12.2[c]
IIB2	3.8 ± 1.2	10.9 ± 2.0[c]	25.1 ± 7.2[c]	35.6 ± 7.4[c]
IIE1	49.8 ± 9.6	74.7 ± 15.2[d]	78.3 ± 17.6[d]	87.9 ± 16.2[c]

[a] Imaoka and Funae (1991).
[b] Unit for changes in the levels of cytochrome P450 is expressed as picomoles of cytochrome P450 per milligram of protein. Values are expressed as means ± SD.
[c] Statistically significant different from control, $p < 0.01$.
[d] Statistically significant different from control, $p < 0.05$.

cumene. Toluene, ethylbenzene, and cumene are clearly more acutely toxic to rats than n-butylbenzene via the oral route. However, there is no clearly common target organ(s) among n-butylbenzene and the three potential surrogates from short-term exposure studies, however, such information may be considered during the weight-of-evidence (WOE) step (Step 7) to facilitate selection of best potential surrogates.

In order to rank and choose the final surrogate, a WOE approach was applied. This WOE approach can be based on (1) a common biological response, similar physiochemical properties, endpoint(s), toxic effect(s), or MOA, (2) metabolites or precursors (note: surrogates belonging to this type may have more weight if the surrogate is a major metabolite that is responsible for the ultimate toxicity), and (3) known dose–response curves based on the TEF or RPF approach among the potential surrogates and chemical of concern.

For n-butylbenzene, a comparison of the chronic target organ(s), critical effects, and effect levels used to identify the PODs for toluene, ethylbenzene, and cumene was made in the previous assessment prior to the availability of the Izumi et al. (2005) study (see Table 5). The liver and/or kidneys appear to be the common target organ(s) following long term oral exposure to these three potential surrogates, and the critical effects appear to occur at similar dose levels. Based on the structural similarity, ethylbenzene is the most similar structural surrogate (70%) followed by cumene (67.7%) and toluene (50%). Similarly, based on the metabolism profiles presented earlier (see Tables 3 and 4), a ranking based on the absorption and elimination data and CYP induction compared to n-butylbenzene can be generalized as ethylbenzene > cumene > toluene. At this point, all the three potential surrogates were considered suitable as the final surrogate for deriving a screening toxicity value for n-butylbenzene. However, in the next step (Step 8), the surrogate with the highest similarity score, most similar metabolic profile, and/or most conservative toxicity value would be chosen.

Overall, ethylbenzene is considered the best surrogate in terms of both structural (highest structural similarity [70%; Table 1] and metabolic surrogacy [see Tables 2 and 3]). In addition, ethylbenzene is the surrogate with the lowest POD value (97.1 mg/kg-day) and is the more acutely lethal than n-butylbenzene (highest mortality incidence in rats; Table 5). The toxicity of short-chain alkylbenzenes does not appear to be a function of MW (see Tables 3–5), thus adjustment of the POD does not seem appropriate. In conclusion, ethylbenzene can serve as a surrogate for n-butylbenzene via the oral route, and the surrogate POD for n-butylbenzene was identified as 97.1 mg/kg-day.

In addition to our surrogate approach, we have also applied the Threshold of Toxicological Concern (TTC) (Munro et al., 1996) approach in this manuscript with some caution in mind: the approach has been applied to mostly food contact materials, flavoring agents and genotoxic contaminants in pharmaceuticals; it has not

Table 5
Toxicity values of n-butylbenzene and potential surrogates.

Chemical	Rat LD50[a] (mg/kg)	Mortality[b]	Short-term target organ	Longer-term target/critical effect	POD[c] (mg/kg-day)	DSSTox[d] similarity score
Toluene	636	3/10	CNS	Increased kidney weight (US EPA, 2005)	238	50
Ethylbenzene	3500	7/10	Liver, kidney, ureter, and bladder	Liver and kidney toxicity (US EPA, 1991)	97.1	70
Cumene (isopropylbenzene)	1400	6/10	Gastrointestinal tract	Increased average kidney weight in female rats (US EPA, 1997)	110	67.7
n-Butylbenzene	4300[e]	2/10	Lungs, thorax, blood	Increased incidences of hepatocellular hypertrophy and increased liver weight in F0 and F1 male rats (Izumi et al., 2005)[f]	137[f]	100[f]

[a] ChemIDplus (2010).
[b] Mortality in fasted rats following single gavage dose of 2.5 ml in olive oil; (Gerarde, 1959).
[c] POD can be based on NOAEL, LOAEL, or BMDL; see www.epa.gov/iris for details.
[d] DSSTox (2010).
[e] The lethality is considered as a LDlo; (Gerarde, 1959).
[f] A recent two-generational reproductive study in rats; results and toxicity values were unknown at time of the SAR-based assessment.

been broadly applied to environmental chemicals. We used n-butylbenzene as an input into the ToxTree module within the OECD Toolbox and found out that it has been assigned a "Low" or "Class I" category with 5th percentile of NOAEL of 3 mg/kg-day. However, the experimental NOAEL is 100 mg/kg-day, which is about 33 times higher than the predicted. In the context of risk assessment and potential clean-up and regulatory considerations, this overestimation of NOAEL (which may serve as a potential point of departure) may not be appropriate. This finding is consistent with the draft opinion by SCHER/SCCP/SCENIHR (2008) that "the application of [TTC] in terms of risk assessment for safety evaluation of a chemical is dependent on the quality, quantity, and relevance of the underlying toxicity database, and a reliable estimation of the exposure to the chemical in the respective field of application." Hence, without a proper exposure assessment (beyond the scope of this manuscript), it is inappropriate to apply TTC to our environmental chemical of concern.

3.3. Demonstration of the tiered surrogate approach

After the surrogate assessment was carried out, a recent rat two-generational reproductive oral study for n-butylbenzene was located (Izumi et al., 2005). There is also a supporting two-generational reproductive study by Yamasaki et al. (2005). No other subchronic- or chronic-duration oral studies have been located. The Izumi et al. (2005) study serves to validate the surrogate toxicity for n-butylbenzene and provides an opportunity to evaluate the tiered surrogate approach. Both studies indicate lack of apparent reproductive toxicity via oral exposure to n-butylbenzene, but the Yamasaki et al. (2005) study is only a review of nine hydrocarbons, and is not a comprehensive study on n-butylbenzene with a focus on endocrine disrupting activity without histopathological or clinical chemistry data.

Izumi et al. (2005) conducted a two-generation reproductive toxicity study of n-butylbenzene via the oral route in Crj:CD(SD) IGS rats. Although it was primarily designed by the study authors to detect potential endocrine disrupting effects, the study is well designed and is appropriate for deriving subchronic and chronic toxicity values, because the study examined several endpoints including body and organ weights as well as histopathology and some clinical chemistry parameters. The study was administered by gavage at dose levels of 0, 30, 100, and 300 mg/kg-day to rats (24 males and 24 females per group) over 2 generations. Only treatment-related histopathological changes were observed in the liver (hepatocellular hypertrophy) and kidneys (hyaline droplets) of the F0 and F1 male parent animals. The highest dose level of 300 mg/kg-day, which shows clear histopathological changes and increased organ weight, is identified as a LOAEL. The NOAEL is 100 mg/kg-day. Benchmark dose (BMD) modeling of the F0 hepatocelullar hypertrophy resulted in BMD_{10} and $BMDL_{10}$ of 266 and 162 mg/kg-day, respectively. The BMD_{10} and $BMDL_{10}$ derived for the F1 hepatocelullar hypertrophy are 245 and 137 mg/kg-day, respectively, indicating a more sensitive response occurred in F1 generation. The $BMDL_{10}$ of 137 mg/kg-day was selected as the POD (see http://hhpprtv.ornl.gov/issue_papers/Butylbenzenen.pdf for detail).

In conclusion, the surrogate chronic POD for n-butylbenzene was determined to be 97.1 mg/kg-day from ethylbenzene based on similar target organs (liver and/or kidney) among the potential surrogates, toxic effects (histopathologic changes in liver and kidney), metabolism profile, and CYP induction. Notably, this value is comparable to the chronic POD (137 mg/kg-day) derived based on the new experimental results by Izumi et al. (2005); the difference between the predicted vs. experimental POD is only ⌋ 1.4-fold. This latter POD is also based on liver toxicity (hepatocellular hypertrophy) accompanied by increased liver weight. This consistency between the estimated (surrogate) and actual PODs demonstrates the utility of this tiered surrogate approach (decision tree in Fig. 1). This test study indicates that for this class of chemical (i.e., alkylbenzenes), the surrogate approach presented here provided a reasonably well-predicted risk value for a chemical of concern with limited toxicity data. However, it is worth noting that this approach only focused on the information from structure, metabolism and available toxicity data. As noted in Wu et al. (2010) study, other considerations could also play an important role in the SAR-based assessment. For example, details on reactivity, electrophiles, nucleophiles, conditions of chemical reactions (pH, S_N1 vs. S_N2, etc.) could also affect the ultimate toxicity. Therefore, for a chemical outside of the alkylbenzene class to be considered as a potential surrogate, further considerations on this additional information might be critical.

4. Discussion

The manuscript has systematically detailed the use of a surrogate approach without any commercial (Q)SAR or expert systems software programs to derive a surrogate toxicity value as part of human health risk assessment. The tiered surrogate approach presented here is flexible and can be tailored for any chemical of concern, and the approach prevents the chemicals of concern with limited or no toxicological data from being assigned "zero" or no toxicity values (such chemicals may be subsequently not considered at a contaminated site). At least a rough estimate can be determined for prioritizing most toxic chemicals for further clean-up or precaution. Although there is a greater uncertainty

by using the surrogate approach, the predicted value gives some guidance on the toxicity potential of a chemical of concern by ranking relative risks of individual chemicals present at a site to determine if the risk developed from the associated exposure to the chemical of concern at the specific site is likely to be a significant concern in the overall cleanup decision. Further experimental data may be required for verification, if the chemical of concern is the primary driver in making cleanup decisions.

Applying a tiered surrogate approach to human health risk assessment has been demonstrated in this manuscript. It shows that it is possible to estimate a toxicity value for long-term exposure based on carefully selected surrogates (e.g., structural, metabolic, and toxicity-like) with transparent rationales and professional judgments. Specifically, a POD can be estimated for a chemical of concern, and the POD coupled with an appropriate composite UF can be further considered as a reference value for screening purposes for chemicals of concern at contaminated sites.

In the tiered surrogate approach, three main types of surrogates were used which can be searched independently or sequentially based on the availability and type of data. In addition, a thorough test case study (n-butylbenzene) was performed to show that this methodology is scientifically justified (only ⌟ 1.4-fold difference in identified PODs between the surrogate and traditional approaches). The test case study shows that our rationale for predicting target organs (liver and/or kidney) and associated toxic effects was indeed appropriate. This indicates that the predictive uncertainty based on this surrogate approach may not be a serious concern, but further work is needed to quantify predictive uncertainty associated with using the surrogate approach. We understand that one test case study is not enough to justify the validity and utility of this surrogate approach; however, it represents the first step in demonstrating the proper use of a surrogate approach for predicting a toxicity value that may have regulatory implications. In the future, we hope to perform more test (validation) case studies when experimental data become available.

To ensure transparency and reproducibility, only publicly available software programs were used in this manuscript. We recognize the possibility that some commercial software programs may provide better results in terms of looking for surrogates (structural, metabolic or activity-like) with built-in expert judgment and/or validated (Q)SAR models. The fundamental issue is still driven by the availability (or lack thereof) of repeated toxicity data for any potential hits (only hits with repeated dose information can be considered as potential surrogates). This limitation could potentially be addressed by designing and building a centralized database with all available peer-reviewed and vetted toxicity data (e.g., IRIS) and a built-in search engine. Furthermore, some parameters such as reactivity and other chemical reactions as described by Wu et al. (2010) are not explicitly accounted for. We understand how chemical reaction is directly related to toxicokinetics, that is, how the organism body may modify the chemical. Characterizing the chemical's reactivity may also be a key for the suitability of potential surrogates. However, because the test case study has some direct toxicokinetic characteristics, that may not be a problem when a chemical's reactivity and other parameters are not fully characterized. It is strongly encouraged to provide such information, especially those properties that could potentially affect the chemical's bioavailability and potential harm.

A MW adjustment is only necessary when a mechanism of action is elucidated and when molecular targets have been identified. It is a common misconception that a MW adjustment must be applied indiscriminately for the use of any surrogate approach when there is no mechanistic information (down to the molecular level). This misconception stems from the practice of pharmaceuticals when one applies a SAR to a series of congeners with a common and elucidated molecular mechanism (e.g., tamoxifen vs. substituted tamoxifens binding to estrogen receptor-alpha [ERa]; Robertson et al., 1982). In general, a consideration of applying a MW adjustment is not necessary if the MW of a potential surrogate is less than a factor of two in comparison to the MW of chemical of concern (i.e., no significant difference in final toxicity value with or without the molecular adjustment after applying all appropriate uncertainty factors). If the MWs of potential surrogates are >2-fold than the MW of the chemical of concern, then a common mechanism of action or stoichiometric reaction should be elucidated prior to the molecular-weight adjustment, because the MW adjustment would result in a >2-fold difference in term of final toxicity values.

The surrogate approach works reasonably well for the test case presented in this manuscript; however, there is still some uncertainty that the surrogate approach may or may not work with other chemical classes or groupings by a predefined criterion (e.g., similar toxic effect/outcome). Future work will include other chemical classes or groupings that will further validate the utility of this surrogate approach. We also envision that other types of high-throughput approaches may be used in conjunction to shed some light in terms of MOA and other relevant toxicity information (i.e., temporal, target cell-line/organ/tissue, etc.) that would strengthen the confidence of the toxicity value predicted by the surrogate approach. These high-throughput and in vitro data may fill in some data gaps that are currently missing in the surrogate approach. Perhaps, one may group chemicals based on the common adverse outcomes that go beyond the chemical class or structural analogs (e.g., congeners). Another grouping or classification approach can also be based on genomic profiles or toxicity pathways. It is strongly encouraged to lay out all rationales that are based on the available information and to note any uncertainty due to a lack of toxicity data on the surrogate itself.

There are five areas of uncertainty that require UFs applied to the POD for extrapolation to the low-dose region and human population: (1) interspecies (animal-to-human), (2) intraspecies (susceptible human subpopulation), (3) subchronic-to-chronic, (4) LOAEL-to-NOAEL, and (5) database deficiencies. The first four areas of uncertainty are typically assumed as the same as for the chemical of concern based on the final surrogate unless there is information on the chemical of concern to suggest otherwise. Typically, the database UF of the surrogate is assumed to be the same as the database UF for the chemical of concern based on the same sensitive endpoint and effect, and a statement should be clearly stated for such an assumption. However, if a different endpoint and associated effect is proposed (other than the most sensitive endpoint and effect of the surrogate), a full database UF is warranted for the chemical of concern. Because some assumptions are based on the suspected (surrogate) target organs and critical effects of the potential surrogates, there is an inherently greater uncertainty in the surrogate approach that cannot assure that such effects and endpoints are the most sensitive endpoints/effects for the chemical of concern in terms of overall health protection. For example, the chemical of concern may have neurological or immunological endpoint/indication as the most sensitive endpoint that was not examined among the potential surrogates. Therefore, it is important to continue work to accumulate knowledge from unexpected "outliers" in order to quantify the surrogate uncertainty, specifically accounting for the uncertainty associated with extrapolation when the database is incomplete (e.g., lack of two-generation reproductive or developmental studies). Although there could be well-characterized database uncertainty for the potential surrogates, one may still apply a full database UF to account for unknown and unaccountable database deficiencies of the chemical of concern itself.

A detailed framework for conducting a tiered surrogate approach for predicting a POD is presented and demonstrated in this manuscript. Careful considerations from all sources of chemical and biological/toxicological information have been incorporated

into the final WOE approach to select the most appropriate surrogate, and its toxicity value(s) is suggested as the surrogate toxicity value(s) for a chemical of concern. This surrogate approach has built-in flexibility and is dynamic in that it can accommodate various tools and technologies beyond the current SAR-based methods. Typically, one does not need to narrow the scope of search for potential metabolic surrogates, and the search can be done in parallel or in tandem with the structural surrogates. For the test case study, the search for potential metabolic surrogates was done in tandem with the structural surrogates because there is chemical class-specific information that these two surrogates types are related and likely to overlap. In other scenarios, it is encouraged to conduct an independent search for metabolic surrogates that may not have any structural similarity with the chemical of concern. Finally, we envision that the flexible surrogate approach can also take advantage of the current high-throughput technologies by utilizing information from genomics and in vitro assays that can strengthen the argument of purported target organs and associated toxic effects. Future work will include several case studies demonstrating the versatility and utility of this approach. Additional work may include a hybrid approach that utilized both SAR and genomics, combining the best of both chemical and biological worlds.

Human health risk assessment includes core components of hazard identification, dose–response assessment, and exposure assessment (NRC, 1983). The outcome of this assessment paradigm is a risk characterization that facilitates the subsequent management of risks in various environments (e.g., water, air). Thus, in practice, the mitigation of environmental risks is dependent upon the availability and transparent communication of data that informs the core components of the risk assessment paradigm. The surrogate methodology presented here illustrates an approach that enables hazard identification and dose–response assessment for potential environmental chemical risks when the lack or virtual absence of data may have previously inhibited assessment. Exposure assessment and risk characterization are needed to complete a full risk assessment.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.yrtph.2012.02.006.

References

Ashby, J., 1985. Fundamental structural alerts to potential carcinogenicity or noncarcinogenicity. Environ. Mutagen. 7 (6), 919–921.

Bodor, N., 1999. Recent advances in retrometabolic design approaches. J. Control. Release 62 (1–2), 209–222.

ChemIDplus (Chemical Identification File), 2010. ChemIDplus database. <http://sis.nlm.nih.gov/chemical.html > Accessed on 7/6/2010.

Congiu, A., Pozzi, D., Esposito, C., Castellano, C., Mossa, G., 2004. Correlation between structure and transfection efficiency: a study of DC-Chol–DOPE/DNA complexes. Colloids Surf. B Biointerfaces 36 (1), 43–48.

Cronin, T.D., Enoch, S.J., Hewitt, M., Madden, J.C., 2011. Formation of mechanistic categories and local models to facilitate the prediction of toxicity. ALTEX 28 (1), 45–49.

DSSTox (Distributed Structure-Searchable Toxicity), 2010. DSSTox database. <http://actor.epa.gov/actor/faces/DSSTox/Home.jsp > Accessed on 7/6/2010.

El Masry, A., Smith, J., Williams, R., 1956. Studies in detoxication. 69. The metabolism of alkylbenzenes: n-propylbenzene and n-butylbenzene with further observations on ethylbenzene. Biochem. J. 64 (1), 50–56.

Gerarde, H., 1959. Toxicological studies on hydrocarbons. III. The biochemorphology of the phenylalkanes and phenylalkenes. AMA Arch. Ind. Health 19 (4), 403–418.

Gerarde, H., Ahlstrom, D., 1966. Toxicologic studies on hydrocarbons: XI. Influence of dose on the metabolism of mono-n-alkyl derivatives of benzene. Toxicol. Appl. Pharmacol. 9 (1), 185–190.

Hendersen, Y., Haggard, H.W., 1943. Noxious Gases. Reinhold Publishing Corp, New York, pp. 221–222.

Imaoka, S., Funae, Y., 1991. Induction of cytochrome P450 isozymes in rat liver by methyl n-alkyl ketones and n-alkylbenzenes. Effects of hydrophobicity of inducers on inducibility of cytochrome P450. Biochem. Pharmacol. 42, Suppl. S143–50.

Izumi, H., Kimura, E., Ota, T., Shimazu, S., 2005. A two-generation reproductive toxicity study of n-butylbenzene in rats. J. Toxicol. Sci. 30 (Special Issue), 21–38.

Miller, R.R., Hermann, E.A., Landvardt, P.W., McKenna, M.J., Schwetz, B.A., 1983. Comparative metabolism and distribution of ethylene glycol monomethyl ether and propylene glycol monomethyl ether in male rats. Toxicol. Appl. Pharmacol. 67 (2), 229–237.

Miller, R.R., Hermann, E.A., Young, J.T., Calhoun, L.L., Kasti, P.E., 1984. Propylene glycol monomethyl ether acetate (PGMEA) metabolism, disposition, and short-term vapor inhalation toxicity studies. Toxicol. Appl. Pharmacol. 75 (3), 521–530.

Mizunuma, K., Kawai, T., Yasugi, T., Horiguchi, S., Iwami, O., Ikeda, M., 1992. In vitro hydrolysis of methyl acetate, a limitation in application of head-space gas-chromatography in biological monitoring of exposure. Toxicol. Lett. 62 (2–3), 247–253.

Munro, I.C., Ford, R.A., Kennepohl, E., Sprenger, J.G., 1996. Correlation of structural class with No-Observed-Effect Levels: a proposal for establishing a threshold of concern. Food Chem. Toxicol. 34, 829–867.

Nagano, K., Nakayama, E., Koyano, M., Oobayashi, H., Adachi, H., Yamada, T., 1979. [Testicular atrophy of mice induced by ethylene glycol mono alkyl ethers (author's transl)]. Sangyo Igaku [Jpn J. Ind. Health] 21:29–35. (English translation submitted under TSCA; NTIS No. FYI-OTS-038-0105; pp. 76–85).

National Academy of Sciences (NAS), 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. National Academies Press, Washington, DC. <http://www.toxicology.org/pm/Krewski_Dec09.pdf >.

National Academy of Sciences (NAS), 2008. Science and Decisions: Advancing Risk Assessment (NAS Final Report). National Academies Press, Washington, DC.

NRC (National Research Council), 1983. Risk Assessment in the Federal Government: Managing the Process. NAS Press, Washington, DC.

NTP (National Toxicology Program), 2006. Tox-48 – NTP technical report on the comparative toxicity studies of allyl acetate, allyl alcohol, and acrolein. Online. http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox048.pdf .

OECD, 2007. OECD Environment Health and Safety Publications. Series on Testing and Assessment No. 80. Guidance on Grouping of Chemicals. Online. <http://www.oecd.org/officialdocuments/displaydocumentpdf?cote=env/jm/mono%282007%2928&doclanguage=en >.

Research Triangle Institute. 1989. Metabolism, disposition and pharmacokinetics of cumene in F-344 rats following oral, IV administration or nose-only inhalation exposure. Report No. RTI/4353-01F. CMA Reference No. CU-5.0-PK-RTI.

Robertson, D.W., Katzenellenbogen, J.A., Hayes, J.R., Katzenellenbogen, B.S., 1982. Antiestrogen basicity–activity relationships: a comparison of the estrogen receptor binding and antiuterotrophic potencies of several analogues of (Z)-1, 2-diphenyl-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1-butene (tamoxifen, Nolvadex) having altered basicity. J. Med. Chem. 25 (2), 167–171.

Sanderson, D.M., Earnshaw, C.G., 1991. Computer prediction of possible toxic action from chemical structure; the DEREK system. Hum. Exp. Toxicol. 10 (4), 261–273.

SCHER/SCCP/SCENIHR, 2008. Draft opinion on the use of the Threshold of Toxicological Concern (TTC) approach for the safety assessment of chemical substances. Online. <http://ec.europa.eu/health/ph_risk/committees/documents/sc_o_001.pdf >.

Seńczuk, W., Litewka, B., 1976. Absorption of cumene through the respiratory tract and excretion of dimethylphenylcarbinol in urine. Br. J. Ind. Med. 33 (2), 100–105.

Tada, O., Nakaaki, K., Fukabori, M., 1974. [The method of evaluating the exposure to methanol and methyl acetate.] Rogo Kagaki [J. Sci. Labour]. 50(4),239–245 (Japanese; Abstract in English) (also cited in US EPA, 1986).

Tambo, S., 1973. [Toxicity hazards of paint thinners with particular emphasis on the metabolism and toxicity of acetate esters.] Nichidal Igaku Zasshi 32, 349–360 (Japanese; Abstract in English) (also cited in US EPA, 1986).

Tong, W., Welsh, W.J., Shi, L., Fang, H., Perkins, R., 2003. Structure–activity relationship approaches and applications. Environ. Toxicol. Chem. 22 (8), 1680–1695.

US EPA, 1993. Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons. US Environmental Protection Agency, Environmental Criteria and Assessment Office, Cincinnati, OH. NTIS PB94-116571. <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=49732 >.

US EPA, 2000. Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. US Environmental Protection Agency, Risk Assessment Forum, Washington, DC. EPA/630/R-00/002. <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=20533 >.

US EPA. 2000. Benchmark Dose Technical Guidance Document. Risk Assessment Forum, Washington, DC. External Review Draft. EPA/630/R-00/001.

US EPA, 2005, Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/P-03/001F. Federal Register 70(66):17765–17817. <http://www.epa.gov/raf/publications/pdfs/CANCER_GUIDELINES_FINAL_3-25-05.PDF>.

US EPA, 2010. Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures (External Review Draft). US Environmental Protection Agency, Washington, DC, EPA/635/R-08/012A, 2010.

van den Broek, L.A., Lázaro, E., Zylicz, Z., Fennis, P.J., Missler, F.A., Lelieveld, P., Garzotto, M., Wagener, D.J., Ballesta, J.P., Ottenheijm, H.C., 1989. Lipophilic analogues of sparsomycin as strong inhibitors of protein synthesis and tumor growth: a structure–activity relationship study. J. Med. Chem. 32 (8), 2002–2015.

Wu, S., Blackburn, K., Amburgey, J., Jaworska, J., Federle, T., 2010. A framework for using structural, reactivity, metabolic and physicochemical similarity to evaluate the suitability of analogs for SAR-based toxicological assessments. Regul. Toxicol. Pharmacol. 56 (1), 67–81.

Yamasaki, K., Takahashi, M., Yasuda, M., 2005. Two-generation reproductive toxicity studies in rats with extra parameters for detecting endocrine disrupting activity: introductory overview of results for nine chemicals. J. Toxicol. Sci. 30, Spec. No. 1–4.